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20-Oxopregnacalciferols: Vitamin D Compounds That Bind The Progesterone Receptor

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Summary: 20-Oxopregnacaliferols and 19-nor-20-oxopregnacalciferol were prepared from calciferol 22-aldehydes by an oxygenation procedure.

RU 486[1] (a 19-nor-steroid, developed in the 1980's by Roussel-UCLAF researchers) has strong anti-progesterone and anti-glucocorticosteroid activities. When used in conjunction with synthetic prostaglandins, it terminates pregnancy, which accounts fo its wide interest. In addition, it has great potential as an anti-glucocorticoid and anti-estrogen agent.[2,3]

Many analogs have been prepared,[4] all of which share with RU 486 the intact steroid A ring of progesterone with the conjugated 3-oxo-4-ene moiety. This feature is assumed to be responsible for binding to the progesterone receptor (PR).

In our ongoing effort to examine the many different aspects of the vitamin D molecule,[5] we describe here vitamin D analogs with the CD ring of progesterone but with the 3β-hydroxycyclohexane A-ring and double bond system, characteristic of vitamin D. Because it is a composite of vitamin D and progesterone, we affectionately term this class of compounds "the vitamin D mermaids". The compounds prepared were 20-oxopregnacalciferol,[6] 1α-hydroxy-20-oxopregnacalciferol and 19-nor-1α-hydroxy-20-oxopregnacalciferol and their binding to the PR was examined.[7]

One of these, 20-oxopregnacalciferol was synthesized earlier for calcemic studies using a classical multi step procedure from pregnenolone acetate.[6] The product was obtained in extremely poor yield, and was shown to have no significant calcemic activity.

The oxidation of the Vitamin D conjugated triene system by singlet oxygen and other reagents has been extensively studied. In all of these oxidative reactions the oxygen and light sensitive triene system was destroyed[8]. In the steroid field, Van Rheenen[9] successfully oxygenated branched aldehydes to ketones in the presence of cupric acetate, complexed with 2,2'-bipyridyl or 1,10-phenanthroline as catalyst, the base 1,4-diazabicyclo[2.2.2]octane (DABCO) and DMF as solvent. We were surprised to find that this unusual oxidative method not only did not destroy the oxygen sensitive polyene system, but we successfully synthesized in one step these 20-oxopregnacalciferols (Vitamin D mermaids) from the corresponding and readily

available vitamin D C-22 aldehyde,[5] 1α-hydroxyvitamin D C-22 aldehyde[5] (calciferol C-22 aldehydes) and 19-nor-1α-hydroxyvitamin D C-22 aldehyde[10] (1, 4, 7)

3-t-Butyldimethylsilyloxy-calciferol C-22 aldehydes 1, 4 and their 19-nor analog 7 were dissolved in DMF, protected from light, and air was bubbled through the solution (1 h at 40°C, RT 22 h, $CuAc_2$-2,2'-bipyridyl complex, DABCO) to give in 60-65% yield the desired 3-t-butyldimethylsilyloxy-20-oxo analogs 2, 5, 8 (with recovery of unconverted aldehyde). Deprotection with tetrahydrofuran-acetic acid-water (3:1:1) (40°C for 3 h, RT for 22 h) gave in 65-70% yield the 20-oxopregnacalciferols 3, 6 and the 19-nor analog 9.

All three compounds were examined for binding to the PR. Cytosolic extracts from MCF-7 cells were incubated with ^{3}H-R 5020 in the presence or absence of a 200 fold excess of the indicated competing compounds. As shown in Table 1. compound 3 competes effectively with the binding of R 5020 to the PR but to a lesser extent than R 5020 or RU 486. Of the three 20-oxo analogs, only 20-oxo-pregnacalciferol (3) bound to the PR, while the 1α-hydroxy analogs 6 and 9 did not.

Additional biological studies are in progress, i.e. determination if the PR binding compounds are agonists or antagonists of progesterone. None of the above compounds had any calcemic activity nor did they bind to the vitamin D receptor. Further, 3 does not bind to the glucocorticoid receptor.

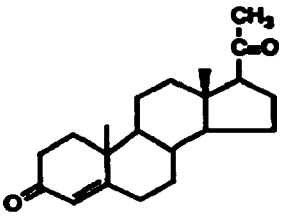
The 20-oxopregnacalciferol is, therefore, the first known vitamin D compound that binds to the PR. It may be selective for progesterone sensitive systems, because it does not bind either the vitamin D receptor or the glucocorticoid receptor.

Table 1.BINDING OF 20-OXOPREGNACALCIFEROL WITH THE PROGESTERONE RECEPTOR.

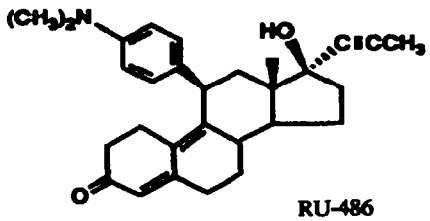
	COMPOUND	TOTAL BOUND(DPM)	% INH.
1-	^{3}H-R5020	4279 ± 202	--
2-	+ R5020	752 ± 93	83%
3-	+ RU 486	941 ± 171	78%
4-	+ 20-OXO-PREGNA-CALCIFEROL.	1976 ± 190	54%

NOTES:

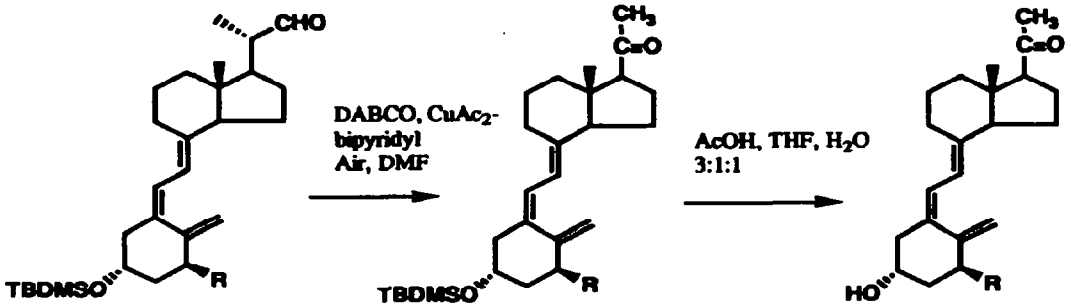
1- ALL COMPETITIVE COMPOUNDS WERE PRESENT AT 200X OF THE ^{3}H-R5020.
2- THE SOURCE OF THE PROGESTERONE RECEPTOR WAS FROM MCF-7 CELLS INDUCED WITH ESROGEN FOR 12 HOURS.
3- THE BINDING OF THE COMPOUNDS WITH THE RECEPTOR WAS ALLOWED TO PROCEED FOR 14 HOURS ON ICE BEFORE PROCESSING.



progesterone



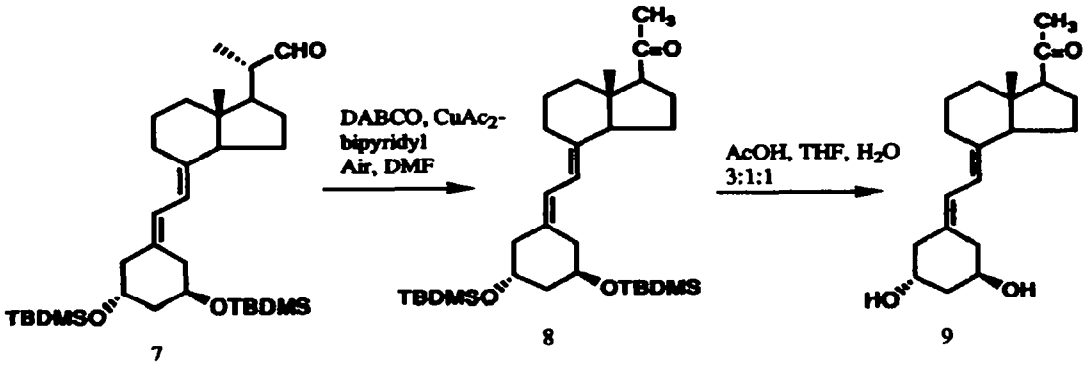
RU-486



1. R=H
4. R=OTBDMS

2. R=H
5. R=OTBDMS

3. R=H
6. R=OH



7

8

9

Analytical Data: All NMR in $CDCl_3$ at 400 or 500 MHz, All MS, EI, 70 ev.

3. UV (in EtOH) λ_{max}: 264 nm, λ_{min}: 228 nm.
   ^1H NMR δ: 0.51 (3H, s, 18-CH_3), 2.13 (3H, s, 21-CH_3), 4.20 (1H, m, 3α-H), 4.45 (1H, m, 1β-H), 4.98 (br s, 19E-H), 5.33 (1H, br s, 19Z-H), 6.04 (1H, d, J=11.3 Hz, 7-H), 6.36 (1H, d, J=11.3 Hz,6-H), MS m/z (rel. int.), 330 (M^+, 31), 312 (21), 183 (95), 134 (100).
6. UV (in EtOH) λ_{max}: 264 nm, λ_{min}: 228 nm.
   ^1H NMR δ: 0.51 (3 H, s, 18-CH_3), 2.13 (3H, s, 21-CH_3), 3.95 (1H, m, 3α-H), 4.81 (br s, 19E-H), 5.06 (1H, br s, 19Z-H), 6.06 (1 H, d, J=11.2 Hz, 7-H), 6.22 (1H, d, J=11.2 Hz, 6-H), MS m/z (rel. int.), 314 (M^+, 23), 296 (2.4), 281 (15), 271 (1.5), 253 (4.6), 136 (33), 118 (73), 43 (100).
9. UV (in EtOH) λ_{max}: 243, 251.5, 261 nm
   ^1H NMR δ: 0.51 (3H, s, 18-CH_3), 2.14 (3H, s, 21-CH_3), 4.06 (1H, m 3α-H), 4.13 (1H, m, 1β-H), 5.88 (1H, d, J=11.3 Hz, 7-H) 6.29 (1H, d, J=11.3 Hz, 6-H). MS m/z (rel. int), 318 (M^+,85), 300 (5), 282 (2), 275 (35), 239 (41), 133 (100), 95 (100).

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